

## The Influence of Ovarian Fluid pH of Stripped Unfertilized Channel Catfish, *Ictalurus punctatus*, Eggs on the Hatching Success of Channel Catfish ♀ x Blue Catfish, *Ictalurus furcatus* ♂, Hybrid Catfish Eggs

NAGARAJ G. CHATAKONDI<sup>1</sup> AND EUGENE L. TORRANS

USDA-ARS Catfish Genetics Research Unit, National Warmwater Aquaculture Center, P.O.  
Box 38, Stoneville, Mississippi 38776, USA

**Abstract.** – This study was designed to determine the effect of ovarian fluid pH of stripped unfertilized channel catfish, *Ictalurus punctatus*, eggs on fertilization and hatch rate of channel catfish ♀ x blue catfish, *Ictalurus furcatus* ♂, hybrid catfish eggs. A significant correlation was established between ovarian fluid pH of stripped channel catfish eggs and hybrid embryo hatch rate ( $R^2 = 0.75$ ,  $P = 0.01$ ), suggesting ovarian fluid pH of stripped catfish eggs prior to fertilization can be predictive of the hatching success of hybrid catfish embryos. These data were used to categorize pH of stripped eggs: pH <7.0 as “low pH eggs,” pH 7.0–7.4 as “medium pH eggs,” or pH >7.4 as “high pH eggs” quality eggs. The range in percent hatch rate for these pH categories was <15%, 15–30%, and >30%, respectively. Higher calcium concentrations during incubation do not appear to improve hatching success of “low pH eggs.” The predictive model presented herein describes a quick method for evaluating stripped catfish eggs for hybrid fry production in catfish hatcheries.

Production of channel catfish, *Ictalurus punctatus* ♀ x blue catfish, *Ictalurus furcatus* ♂, hybrid catfish is rapidly increasing in the USA, largely because of the superior performance of hybrid catfish compared with the commonly raised channel catfish and increased availability of hybrid catfish fingerlings. Hybrid fry production has increased substantially in the last 10 yr from a mere 1 million fry (2001) to over 110 million fry (2011) in commercial catfish hatcheries (personal communication with hybrid catfish producers). Variable and inconsistent hybrid embryos production is still a major problem in hatcheries; hence, there is a need to optimize hybrid fry production protocols to improve efficiency of channel x blue hybrid embryo production.

The term “egg quality” describes the proportion of eggs in an egg batch that successfully complete development to a distinct ontogenetic stage, whereby the survival to hatch is a representative criterion. Variable egg quality is one of the most important constraints to the development of aquaculture (Kjorsvik et al. 1990; Bromage et al. 1992). Poor egg quality, which can stem from parental genetics, diet, stress, poor water quality, or variable egg maturation, and increased handling seen when manually stripping fish (Kjorsvik et al. 1990; Brooks et al. 1997). Alterations in pH of ovarian fluid and high protein levels were observed as indicative of low-quality and overripe egg batches in turbot, *Scophthalmus maximus* (Fauvel et al. 1993). A reduced egg quality may also contribute to lower survival during the early life history stages of teleosts. Unfortunately, there are not many ways to determine egg quality, and in practical hatchery operations, egg quality is revealed only at hatching. If eggs of poor quality are incubated, farmers suffer losses from fungal diseases leading to poor fry production, from the cost of labor, and facilities used to incubate them.

Empirical measurement of egg quality would allow low-quality eggs to be identified and discarded prior to incubation so as to reduce the spread of fungal diseases to healthy eggs. Perhaps more important, objective egg quality measures could be used to assess success of broodfish management practices to improve the efficiency of hatchery output. Hybrid catfish embryo production is a hormone-based

<sup>1</sup> Corresponding author.

synchronization protocol consisting of collecting eggs from stripable, induced channel catfish females, fertilizing them with blue catfish sperm, and incubating fertilized eggs to hatch fry. Stripping eggs from hormone-induced channel catfish female often results in a little blood being mixed with the ovarian fluid. If too much blood is present, this suggests that the eggs have not been ovulated and that the fish is not ready to spawn.

The stage of oocyte maturation at spawning induction has major effects on developmental capacities of subsequently ovulated oocytes (Bobe and Labbe 2010). Hence, egg quality evaluation is of utmost importance, whenever protocols for induced final oocyte maturation (FOM) and ovulation are being developed (Mylonas et al. 1992). Hatchery production can be optimized by starting the production cycle with high-quality eggs that improve egg viability and hatching rates to produce robust embryos with better survival and stress resistance.

Hormonal induction for FOM and ovulation of farmed fish often result in reduced egg quality compared with naturally spawned eggs (Brooks et al. 1997). This may be a consequence of a particular hormone, the dosage or administration protocol, varying stages of maturation (both underripe and overripe), mechanical damage from the stripping process, and also being a few hours younger (post-fertilized), when first handled than pond-spawned eggs. No effective predictive marker or estimator of egg quality exists in channel catfish, even though non-viable unfertilized eggs can be occasionally identified by their whitish color and less glossy appearance. Preliminary research has indicated that pH of the ovarian fluid of stripped channel catfish could provide an empirical measure of egg quality to predict subsequent hatch rate.

Hence, the first objective of this research was to determine the feasibility of using pH of stripped unfertilized eggs to predict fertilization and hatch rates of hybrid catfish. We previously determined that hybrid eggs require a higher calcium hardness concentration than channel catfish (manuscript in review). Therefore, the second objective of this research was

to determine if hatch rates of low-quality eggs could be increased by using a higher than recommended calcium concentration in the hatchery water.

## Materials and Methods

### *Hormone Induction*

Approximately 4-yr-old mature female channel catfish of the "Delta" strain were used in the study. These fish were hand selected based on superior secondary sexual characteristics and stocked at 1000 kg/ha in a freshly filled 0.4-ha pond in March 2010 at the Thad Cochran National Warm Water Aquaculture Center, Stoneville, Mississippi, USA. In June 2010, gravid females were hand selected and placed in individual soft mesh bags that were hung in 10,000-L concrete tanks supplied with flow-through water and air (water temperature = 26.1 C, pH 8.6, and dissolved oxygen >6.0 mg/L). Luteinizing hormone releasing hormone (LHRHa; Western Chemicals, Seattle, WA, USA) was administered in two doses, a priming intraperitoneal injection of 20 µg/kg body weight (BW) followed by a resolving dose of 80 µg/kg BW 15 h later, following the protocols described by Kristanto et al. (2009). Sperm was obtained from pond-raised 6-yr-old D&B strain blue catfish by excising and macerating the testes in 0.85% saline solution.

Ovulation occurred 26–30 h after administration of the resolving dose. At hourly intervals, the mesh bags were slightly lifted above the water and examined for the presence of expressed eggs adhered to the bag, an indication that the female was ready to be stripped. Ovulating females were anesthetized by immersion in a buffered 100 mg/L tricaine methane-sulfonate (MS 222; Argent Laboratories, Inc., Redmond, WA, USA) solution, and the eggs were stripped into a 22.5-cm pie pan greased with a thin layer of vegetable shortening. The stripped eggs from each female were weighed to the nearest 0.1 g.

### *pH of Stripped Eggs and Hatching Success*

The egg mass containing ovulated egg and ovarian fluid was gently mixed with a plastic

spoon, and the *in vitro* pH of ovarian fluid and ovulated eggs was measured with an electrode using an HI 9321 pH meter (Hanna Instruments, Ann Arbor, MI, USA). Ovarian fluid pH was measured throughout the stripping process, and the pH observed at the end of the stripping process was recorded. The pH meter was calibrated on a daily basis with the standard reagents provided by the manufacturer. In the first study, 71 females were strip spawned and pH of the ovarian fluid and eggs was measured throughout the stripping process. Two samples of 400 eggs from each female were weighed, fertilized with blue catfish sperm, activated with hatchery water, and water hardened to obtain an adhesive egg sample. Individual samples were held in 2-mm mesh cups (5 cm diameter  $\times$  15 cm high) in an 80-L aquarium. The eggs were placed 10 cm below the water surface, similar to a depth fertilized eggs suspended in a typical hatching trough. A total of 24, 2-mm<sup>2</sup> mesh were suspended in an aquarium that was supplied with blown air from an airstone and flow-through water (one exchange per hour) at 26.1 C. Five such aquaria were used in the study, and the sixth aquarium housed the remaining 22 cups. Dissolved oxygen and temperature were monitored twice daily, and total ammonia and calcium hardness were measured once daily until hatch. Percent fertilization was expressed as a proportion of the total number of live eggs to the total number enumerated in a sample 24 h after fertilization. Disassociation of the chorion was an indicator of an unfertilized egg. Percent hatch was expressed as a proportion of the total number of live hybrid sac fry to the total number of fertilized eggs.

#### *Egg Quality and Calcium Hardness on Hatching Success*

A second study was conducted in four recirculating aquaria rack systems to evaluate 12 treatments (a combination of three pH levels of the stripped unfertilized channel catfish eggs [ovarian fluid and egg mixture] and four levels of calcium hardness in hatching waters). On June 7, 2010, stripped channel catfish eggs

from 10 females were categorized within one of three subjective ovarian fluid pH ranges: pH <7.0 (6.8–7.0); pH 7.0–7.4 (7.1–7.4), and pH >7.4 (7.8–8.2). Three females were assigned to the first two categories and four females were assigned to the third category of ovarian fluid pH ranges. Eight egg samples (20–25 g) fertilized and water-hardened eggs per female channel catfish were randomly distributed to two 23-L aquaria in each of the four aquarium racks maintained with 25 (22–30), 50 (47–51), 75 (71–80), or 100 (92–105) mg/L of calcium hardness. All the aquaria in a rack system were supplied with air and recirculating water maintained at 26.1 C. The aquarium rack system design was previously described (Small 2006). Each rack system had 16 aquaria, and each tank was independently supplied with recirculated water, air, and a rear-side drain with a removable mesh screen. Each tank was independently fed and drained at a constant rate of 7.5 L/min via an adjustable flow regulator. The reservoir tank was a 100-L heavy plastic tank on the bottom rack with a submersible pump to recirculate the water. Calcium hardness waters were prepared by adding 1.3 mL of commercial-grade calcium chloride for an increase of 1 mg/L of calcium hardness to the well water. The total hardness of well water was 2.3 mg/L. Total ammonia and nitrite levels were below 0.05 mg/L, total alkalinities were 300 mg/L, dissolved oxygen was 7.5–7.8 mg/L (saturation of 93–98% maintained at 26.1 C), and temperature was 25.8–26.6 C.

Testes from two mature blue catfish were mixed to randomize the male effects in the study. The fertilization procedures outlined by Kristanto et al. (2009) were followed in this study, where 2.5 mL of blue catfish sperm solution was used per 100 g of stripped eggs. Eggs were fertilized and water hardened with the test waters, in which they would be incubated by the forced-air method described by Carmichael et al. (1993). Calcium hardness was measured twice daily with a Hach kit model FF-2 with digital titration. Percent fertilization was expressed as a proportion of the total number of live eggs to the total number enumerated in a sample 24 h after fertilization. Disassociation

of the chorion was an indicator of an unfertilized egg. Percent hatch was expressed as a proportion of the total number of live hybrid sac fry to the total number of fertilized eggs.

### *Statistical Analyses*

In the first study, fertilization and hatching success of stripped eggs from 71 females was expressed on a percentage basis and then arcsine transformed before statistical analysis to maintain homogeneity of variance. Statistical significance was determined by analysis of variance (ANOVA) using mixed-model procedures of Statistical Analysis System software version 9.1 (SAS Institute, Inc. 1982). If the differences were significant, means were separated by Tukeys *post hoc* test. The "percent hatch" of <15, 15–30, and >30 of hybrid catfish eggs was used to categorize stripped eggs: pH <7.0 as "low pH eggs," pH 7.0–7.4 as "medium pH eggs," and pH >7.4 as "high pH eggs," respectively. The percent fertilization and hatch data and corresponding ovarian fluid pH of the stripped channel catfish were regressed to establish a relationship between ovarian fluid pH of the stripped unfertilized channel catfish eggs and the percent hatch of hybrid embryos.

In the second study, the data were analyzed using ANOVA in a randomized complete block design with the source of variation associated with the eggs of 10 stripped channel catfish females serving as the blocking factor. The percentage data were arcsine transformed prior to one-way ANOVA analysis. The percent fertilization and percent hatch were analyzed in a  $3 \times 4$  factorial arrangement (Factor 1 = pH of stripped channel catfish eggs and Factor 2 = concentration of calcium carbonate in hatching water). Interaction of both the factors for percent fertilization and percent hatch was also assessed. If the differences were significant, means were separated by Tukeys *post hoc* test. In all statistical comparisons, differences were considered significant at  $P < 0.05$ . Interaction of factors (pH of ovulating fluid and concentration of calcium carbonate in hardness water) for percent fertilization and percent hatch was determined.

### **Results**

In the first study, no relationship was found between percent fertilization and ovarian fluid pH of unfertilized stripped channel catfish eggs from 71 females ( $Y = 11.27 \times \text{pH}$ ,  $R^2 = 0.25$ , and  $P = 0.11$ ). However, a positive linear relationship was established between hatch rate and ovarian fluid pH of the stripped eggs ( $Y = 24.84 \times \text{pH} - 156.2$ ,  $R^2 = 0.75$ ,  $P = 0.01$ , and  $n = 71$ ), suggesting ovarian fluid pH of stripped channel catfish eggs prior to fertilization can be predictive of hatching success of hybrid embryos (Fig. 1). The "percent hatch" criteria were used to categorize pH of stripped eggs: pH < 7.0 as "low pH" eggs, pH 7.0–7.4 as "medium pH eggs," or pH > 7.4 as "high pH" eggs to represent <15, 15–30, and >30 percent hatch of hybrid catfish eggs, respectively.

The least square mean percent fertilization of hybrid eggs with "low pH" eggs was (76.7%), which was lower ( $P = 0.03$ ) than "medium pH" eggs (87.3%) and "high pH" eggs with pH > 7.4 (90.0%). However, the percent fertilization did not differ ( $P > 0.05$ ) between "medium pH eggs" and "high pH eggs" (Table 1). Mean percent hatch of hybrid eggs with "high pH eggs" (41.9%) was higher ( $P = 0.01$ ) than the mean percent hatch of "medium pH" eggs (24.6%), which was also higher ( $P = 0.01$ ) than mean hatch of "low pH eggs" (8.2%).

In the second study, stripped eggs from 10 channel catfish females were categorized based on the "pH of the ovarian fluid" criteria established in the first study. Three females were qualified for assignment to "low pH" and "medium pH" categories each, and four females were assigned to "high pH" category. This study was conducted in four recirculatory aquaria rack systems that were independently maintained at 25, 50, 75, and 100 mg/L of calcium hardness. Twelve treatments (three pH categories and four levels of calcium hardness waters) were assessed for percent fertilization and percent hatch. Eight samples of eggs from a female were randomly distributed to two aquaria per rack system.

The mean percent fertilization was similar among the four levels of calcium hardness for

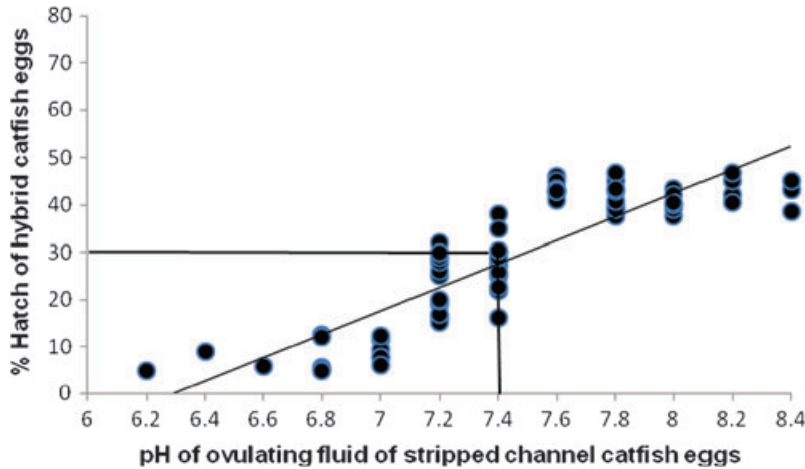


FIGURE 1. Duplicate samples (ca. 400 eggs) from 71 female channel catfish, *Ictalurus punctatus* were fertilized with blue catfish sperm, water hardened, and placed in 2-mm mesh cups of  $5 \times 15$  cm suspended in flow-through aquaria (26.1 C). A linear relationship was established between ovarian fluid pH of the unfertilized channel catfish eggs before fertilization with blue catfish, *Ictalurus furcatus* sperm and percent hatch of channel  $\times$  blue hybrid embryos ( $Y = 24.84 \times \text{pH} - 156.2$ ,  $R^2 = 0.75$ , and  $P = 0.01$ ). The “percent hatch” criteria were used to categorize pH of stripped eggs: pH < 7.0 as “low pH eggs,” pH 7.0–7.4 as “medium pH eggs,” or pH > 7.4 as “high pH eggs” to represent <15, 15–30, and >30 percent hatch of hybrid catfish eggs, respectively.

TABLE 1. Mean  $\pm$  SE<sup>1</sup> of percent fertilization and hatch of channel catfish, *Ictalurus punctatus*  $\times$  blue catfish, *Ictalurus furcatus* hybrid catfish embryos produced with three egg qualities based on ovarian fluid pH of stripped unfertilized eggs of channel catfish.

pH of stripped eggs <sup>2</sup>	n	Fertilization (%)	Hatch (%)
“Low pH” pH < 7.0	14	76.7 $\pm$ 2.6 <sup>a</sup>	8.2 $\pm$ 0.8 <sup>a</sup>
“Medium pH” pH 7.0–7.4	25	87.3 $\pm$ 3.8 <sup>b</sup>	24.60 $\pm$ 1.1 <sup>b</sup>
“High pH” pH > 7.4	32	90.0 $\pm$ 1.1 <sup>b</sup>	41.9 $\pm$ 0.5 <sup>c</sup>

<sup>1</sup>Means within a column with different letters are different ( $P = 0.03$ ) for percent fertilization and ( $P = 0.01$ ) for percent hatch.

<sup>2</sup>Seventy-one stripped channel catfish eggs were graded based on pH either <7.0 as “low pH” eggs, 7.0–7.4 as “medium pH” eggs, or >7.4 as “high pH” eggs, fertilized with blue catfish sperm until hatch in duplicate 2-mm plastic mesh cups ( $5 \times 15$  cm) suspended in 80-L aquaria with flow-through water (26.1 C) and air.

a level of egg quality (Table 2). The percent hatch of hybrid embryos did not differ ( $P > 0.05$ ) among the four levels of calcium hardness assessed in either “low”- or “medium”-quality eggs. If calcium concentration effect was

considered alone, mean fertilization did not differ ( $P > 0.05$ ) between 25, 50, 75, or 100 mg/L. Mean percent hatch of hybrid eggs incubated at 75 mg/L was higher ( $P < 0.05$ ) than 25, 50, or 100 mg/L calcium hardness. Percent hatch of hybrid eggs incubated at 50 mg/L calcium hardness did not differ ( $P > 0.05$ ) with 100 mg/L calcium hardness, but was higher ( $P < 0.05$ ) than 25 mg/L calcium hardness waters.

Interaction of egg quality and calcium hardness for percent fertilization and percent hatch of hybrid embryos incubated at four levels of calcium hardness for three levels of egg quality were not significant ( $P = 0.59$  and 0.29), respectively.

## Discussion

Poor hatch rate is often seen in hybrid catfish hatcheries, which stems largely from inconsistencies in egg quality and suboptimal hatching conditions. Calcium hardness in hatching waters in catfish hatcheries can range from 25 to 100 mg/L, and the minimum concentration for optimal hatching success of hybrid catfish embryos is unknown. Past studies reveal

TABLE 2. Least square means<sup>1</sup> of percent fertilization and percent hatch of channel catfish, *Ictalurus punctatus* x blue catfish, *Ictalurus furcatus* hybrid catfish eggs.

Stripped eggs <sup>2</sup>	Ca conc	Fertilization (%)	Hatch (%)
pH			
7.0	25	84.1 ± 4.7 <sup>a</sup>	5.75 ± 0.99 <sup>d</sup>
	50	85.0 ± 5.3 <sup>a</sup>	6.73 ± 1.1 <sup>d</sup>
	75	87.5 ± 3.5 <sup>a</sup>	10.1 ± 1.6 <sup>d</sup>
	100	85.3 ± 6.0 <sup>a</sup>	8.7 ± 2.2 <sup>d</sup>
7.0–7.4	25	74.3 ± 11.2 <sup>a</sup>	24.2 ± 2.8 <sup>c</sup>
	50	82.5 ± 4.9 <sup>a</sup>	24.5 ± 2.4 <sup>c</sup>
	75	79.4 ± 5.4 <sup>a</sup>	30.8 ± 2.5 <sup>c</sup>
	100	80.5 ± 4.7 <sup>a</sup>	25.5 ± 2.5 <sup>c</sup>
7.4	25	92.0 ± 1.4 <sup>a</sup>	35.5 ± 3.2 <sup>b</sup>
	50	92.8 ± 1.5 <sup>a</sup>	45.9 ± 4.2 <sup>a</sup>
	75	91.8 ± 1.8 <sup>a</sup>	54.4 ± 4.1 <sup>a</sup>
	100	89.5 ± 2.2 <sup>a</sup>	49.1 ± 3.2 <sup>a</sup>
pH groups ( <i>P</i> value)		0.03	0.01
pH < 7.0		76.7 ± 3.2 <sup>a</sup>	7.7 ± 3.9 <sup>c</sup>
pH 7.0–7.4		87.3 ± 2.3 <sup>b</sup>	26.3 ± 3.6 <sup>b</sup>
pH > 7.4		90.0 ± 1.1 <sup>b</sup>	46.1 ± 1.6 <sup>a</sup>
Ca conc groups ( <i>P</i> value)		0.38	0.05
25		80.1 ± 5.3 <sup>a</sup>	21.9 ± 2.1 <sup>a</sup>
50		86.8 ± 2.6 <sup>a</sup>	29.2 ± 2.7 <sup>b</sup>
75		86.3 ± 2.6 <sup>a</sup>	35.3 ± 1.0 <sup>c</sup>
100		85.1 ± 2.7 <sup>a</sup>	28.6 ± 2.3 <sup>b</sup>
pH groups × Ca conc ( <i>P</i> value)		0.59	0.29

<sup>1</sup>Means within a column with different letters are different ( $P < 0.05$ ).

<sup>2</sup>Stripped channel catfish eggs from 10 females were graded based on ovarian fluid pH (pH < 7.0, pH 7.0–7.4, or pH > 7.4) as three pH groups as “low pH,” “medium pH,” or “high pH,” respectively, and eight samples from a female were fertilized with blue catfish sperm, water hardened, and randomly assigned to two 23-L aquaria to an aquarium rack system with 25, 50, 75, and 100 mg/L of calcium hardness (Ca conc) water (26.1 °C).

that hormone-induced fish often ovulate eggs of varying quality largely from variations in brood fish maturity, husbandry, nutrition, and genetics (Kjorsvik et al. 1990; Bromage 1995). Such multifactorial constitution of egg quality makes it difficult to control; therefore, access to a simple tool for evaluating egg quality would be useful in terms of time and cost. There is also a need for estimating egg quality to clarify if the low survival rate during the embryo stage is from initial viability of fry or egg quality.

In our first study, a significant linear relationship ( $R^2 = 0.75$  and  $P = 0.01$ ) was established between pH of the ovarian fluid of stripped catfish eggs and subsequent percent hatching success of hybrid embryos. Our findings concur with earlier reports that observed a significant correlation ( $r = 0.92$  and  $P < 0.001$ ) between ovarian fluid pH and fertilization rate in turbot, which prompted Fauvel et al. (1993) to suggest ovarian fluid pH may be used to assess egg quality. Their studies also confirmed that pH of ovarian fluid declined from 8.1 at ovulation to 7.1 at 10 h post-ovulation, where the eggs were considered to be overripe. In general, ovarian fluid pH was higher than surrounding physiological fluids (blood plasma and vitellus) in turbot. The decrease in ovarian fluid pH during overripening appeared to be linked to an increase in total protein and  $K^+$  content of the fluid, with most poor-quality eggs being associated with an ovarian pH of 8.0 and below. In Cyprinids (Lahnsteiner et al. 1997) as in catfish, ovarian fluid is produced by the epithelium of the ovarian cavity that exhibits a highly secretory activity. There is no ovarian cavity *sensu stricto* in Salmonids, and the ovarian fluid is likely produced by the post-ovulatory ovary, which is known to undergo rapid morphological and probably secretory changes. The relationship between ovarian fluid pH and egg quality could thus result from the coincident changes in characteristics of the ovarian secretions and loss of developmental potential due to egg aging. Blood is mixed with the ovarian fluid owing to the breakage of capillary vessels, generally associated with incorrect hand-stripping technique. Mixing blood with ovarian fluid often reduces the pH of the ovarian fluid of stripped eggs, resulting in lower percent hatch (Ingram 1986).

Our findings suggest that ovarian fluid pH of stripped unfertilized channel catfish eggs was predictive of their viability as hybrid embryos ( $R^2 = 0.75$  and  $P = 0.01$ ) and was consistent with the findings of Fauvel et al. (1993) in turbot, *S. maximus* and Lahnsteiner et al. (1999) in lake trout, *Salmo trutta*. Later, studies by Lahnsteiner (2000) in rainbow trout,

*Oncorhynchus mykiss*, correlated pH, osmolarity, and protein concentration of ovarian fluid to survival to eyed stage. Studies conducted by Wilcox et al. (1984) and Wojtczak et al. (2004) suggested that egg samples characterized by low ovarian fluid pH may not be fertilized because of impaired sperm motility, caused by combination of low pH, high concentration of  $K^+$ , and yolk precipitation. However, caution is required when comparing ovarian fluid pH values reported by different laboratories. Measures of pH will vary depending on the time between egg collection, pH measurement, and on storage temperature during this period. Such progressive change in acidobasic balance of ovarian fluid in stripped eggs is expected when it is removed from the body cavity and placed in contact with air (Aegerter and Jalabert 2004). In this study, percent hatch of hybrid eggs where the pH was 7.6 and higher was more consistent than at lower pH values. Ovarian fluid of low pH is potentially the most detrimental for hatching success of hybrid catfish eggs. Therefore, replacement of the commonly used hatchery water with alkaline buffered solutions (Wojtczak et al. 2006) is recommended to set the fertilizing media to decrease the variations in hatching success caused by the lower pH of ovarian fluid of stripped eggs.

It was also observed that variations of more than 0.5 pH units occurred within the same samples of unfertilized eggs, when measured at the beginning and the end of stripping process. Under suboptimal conditions, strip spawning of channel catfish yields high-quality eggs at the beginning of the stripping process followed by low-quality eggs, blood, and clumps at the end (Chatakondi and Veverica, unpublished results). Separating eggs of high quality and low quality before fertilization may further improve hatching success of hybrid embryos. Results from this study, together with previously published results, suggest that ovarian fluid pH of stripped catfish eggs serves as an indicator of egg quality. However, there is a need to standardize the pH measurement protocol of ovarian fluid in channel catfish and its application as an egg quality indicator under user conditions before one can implement in commercial conditions.

After temperature and dissolved oxygen, calcium is the next most important water quality characteristic in hatchery operations. Apart from being a major cation in natural waters, calcium primarily contributes to osmotic pressure and reduces the hydration of polar organic molecules of the chorion (Maetz 1974). The pioneering work of Tucker and Steeby (1993) led to the practice of supplementing 10 ppm calcium hardness in catfish hatcheries. Based on the results of our recent study (NAJA, accepted manuscript), 75 mg/L calcium hardness appears to be the optimal level to hatch hybrid embryos. We are aware that sets of stripped channel catfish eggs are often lower quality compared with naturally pond-spawned channel catfish eggs because of the hormone treatment and younger age of eggs when handled (post-ovulation). These pools of stripped eggs also contain varying maturational stages and some may have undergone mechanical damage during stripping. Further, poor water hardening reduces egg turgor, which increases susceptibility to mechanical injury and decreases their ability to survive (Ketola et al. 1988). Egg handling procedures, such as transport to the incubation facility, placement into incubators, enumeration, and disinfection, cause mechanical shocks leading to significant egg mortalities (Krise 2001). All these factors have prompted us to advocate higher calcium levels to incubate hybrid embryos.

Our findings confirmed that mean hatching success of hybrid catfish embryos at 75 mg/L calcium hardness waters was higher ( $P = 0.05$ ) than at 25, 50, or 100 mg/L calcium hardness levels (Table 2). However, fertilized eggs incubated at higher calcium hardness levels did not improve mean percent fertilization and mean percent hatch of "low"- or "medium"-quality eggs (Table 2). The predictive egg quality model in this paper is a practical tool for management and incubation of hybrid catfish eggs in catfish hatcheries. Improving efficiency of hybrid catfish embryo production reduces the cost of producing hybrid catfish fry and is likely to result in significant improvement in pond-raised catfish production.

## Management Recommendations:

1. Channel catfish eggs of low quality can also be identified by a simple measure of ovarian fluid pH after stripping but before fertilization. Separation of low-quality eggs from medium- and high-quality eggs reduces the spread of fungal diseases and effectively utilizes hatchery space, labor, and resources to improve the hatching efficiency.
2. As the hatch rate of poor-quality eggs cannot be improved by incubating in higher calcium hardness waters, efforts should be devoted to improve the husbandry, nutritional preparation of broodfish, and to develop effective spawning induction protocols in channel catfish.

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